

John G.K. Williams
Application No.: 09/876,375
Page 2

PATENT

REMARKS

Claims 1-54 are pending in this application and presented for examination.

Applicants hereby elect Group II, drawn to a method for identifying an intact charge-switch NP probe and to an intact charge-switch NP probe, with traverse. Claims readable thereon include claims 18-44. The claims as pending are attached for the Examiner's convenience.

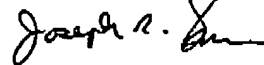
Reconsideration of the restriction requirement is respectfully requested.

Applicants traverse the restriction requirement as the two criteria for a proper restriction requirement have not been met. Under M.P.E.P. § 803, to be proper, the inventions must be independent or distinct; and there must be a serious burden on the Examiner.

Applicants believe Groups I and II should be joined and claims 1-44 examined on their merits. Both independent claims 1 and 18 set forth a sample comprising an intact charge-switch NP probe, an enzyme and an energy field such as an electric field. As such, the requirements for a proper restriction between these two groups has not been met. Accordingly, Groups I and II should be joined and examined together.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



Joseph R. Snyder
Reg. No. 39,381

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
JS:dad (sea)

CCD

WC 9037261 v1

John G.K. Williams
Application No.: 09/876,375
Page 3

PATENT

PENDING CLAIMS

1 1. A method for separating an intact NP probe from a phosphate detectable
2 moiety, said method comprising:

3 a) providing a sample comprising an intact NP probe with a detectable
4 moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe, which
5 produces a phosphate detectable moiety, said phosphate detectable moiety carries a molecular
6 charge which is different than the molecular charge of said intact NP probe; and

7 b) applying an energy field to said sample, thereby separating said phosphate
8 detectable moiety from said intact NP probe.

1 2. The method according to claim 1, wherein said intact NP probe is a
2 charge-switch nucleotide phosphate probe having a detectable moiety on a terminal phosphate.

1 3. The method according to claim 2, wherein said charge-switch nucleotide
2 phosphate is a nucleotide triphosphate (NTP) having a γ -phosphate with a detectable moiety
3 attached thereto.

1 4. The method according to claim 3, wherein said γ -phosphate with a
2 detectable moiety attached thereto is a γ -phosphate with a fluorophore attached thereto.

1 5. The method according to claim 1, wherein said intact NP probe is
2 incorporated on a primer strand hybridized to a target nucleic acid using a polymerase, thereby
3 releasing said phosphate detectable moiety.

1 6. The method according to claim 1, wherein said polymerase is
2 immobilized.

1 7. The method according to claim 1, wherein said energy field is an electric
2 field.

~~COPY~~

John G.K. Williams
Application No.: 09/876,375
Page 4

PATENT

~~COPY~~

1 8. The method according to claim 7, wherein said electric field is a first
2 electric field applied in a transverse direction and a second energy field is applied in an axial
3 direction.

1 9. The method according to claim 8, wherein said second energy field
2 applied in said axial direction is a pressure field.

1 10. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is greater than said intact NP probe.

1 11. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is less than said intact NP probe.

1 12. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is opposite in sign compared to said intact NP probe.

1 13. The method according to claim 1, further comprising c) detecting said
2 phosphate detectable moiety.

1 14. The method according to claim 13, wherein said detection is via a charge
2 coupled device (CCD) camera.

1 15. The method according to claim 13, wherein said detection is via a dye-
2 impregnated polymeric coating on optical fiber sensor.

1 16. The method according to claim 13, wherein said detection is via a
2 photodiode.

1 17. The method according to claim 13, wherein said detection is via a
2 blockade current.

1 18. A method for identifying an intact charge-switch nucleotide phosphate
2 (NP) probe, said method comprising:

~~COPY~~

John G.K. Williams
Application No.: 09/876,375
Page 5

~~CONFIDENTIAL~~

PATENT

3 a) contacting a sample comprising said intact charge-switch NP probe with
4 an enzyme to produce a phosphate detectable moiety; and

5 b) applying an electric field to said sample, wherein said phosphate
6 detectable moiety migrates to an electrode differently than said intact charge-switch NP probe.

1 19. The method according to claim 18, wherein said electrode is an anode.

1 20. The method according to claim 18, wherein said electrode is a cathode.

1 21. The method according to claim 18, wherein either said intact NP probe has
2 a positive molecular charge, or wherein upon cleavage of said phosphate detectable moiety, said
3 phosphate detectable moiety carries a positive charge relative to said intact NP probe.

1 22. The method according to claim 18, wherein said enzyme is selected from
2 the group consisting of a DNA polymerase, a DNA dependent RNA polymerase, a reverse
3 transcriptase, a phosphodiesterase and a phosphatase.

1 23. The method according to claim 18, wherein said intact charge-switch NP
2 probe is a member selected from the group consisting of a nucleotide diphosphate, a
3 deoxynucleotide triphosphate (dNTP), and a nucleotide triphosphate (NTP).

1 24. The method according to claim 23, wherein said deoxynucleotide
2 triphosphate (dNTP) is a member selected from the group consisting of deoxyadenosine
3 triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate deoxythymidine
4 triphosphate and deoxyuridine triphosphate.

1 25. The method according to claim 18, wherein said phosphate detectable
2 moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1 26. The method according to claim 25, wherein upon cleavage of said
2 pyrophosphate fluorophore moiety, said pyrophosphate fluorophore moiety carries a positive
3 charge relative to said intact NTP probe.

~~CONFIDENTIAL~~

John G.K. Williams
Application No.: 09/876,375
Page 6

PATENT

~~copy~~

1 27. The method according to claim 18, wherein said intact NP probe has a
2 positive charge.

1 28. The method according to claim 18, wherein said intact NP probe has a
2 negative charge.

1 29. An intact charge-switch nucleotide phosphate (NP) probe, wherein, upon
2 enzymatic cleavage of said intact charge-switch NP probe to produce a phosphate detectable
3 moiety, said phosphate detectable moiety migrates to an electrode, and intact charge-switch NP
4 probe migrates to the other electrode.

1 30. The intact charge-switch NP probe according to claim 29, wherein either
2 said intact NP probe has a positive molecular charge, or wherein upon cleavage of said
3 phosphate detectable moiety, said phosphate detectable moiety carries a molecular positive
4 charge relative to said intact NP probe.

1 31. The intact charge-switch NP probe according to claim 29, wherein said
2 charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said phosphate
3 detectable moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1 32. The intact charge-switch NP probe according to claim 29, wherein said
2 intact NTP probe has a positive charge.

1 33. The intact charge-switch NP probe according to claim 31, wherein upon
2 cleavage of said phosphate detectable moiety as a pyrophosphate fluorophore moiety, said
3 pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP probe.

1 34. The intact charge-switch NP probe according to claim 29, wherein said
2 NTP probe is a member selected from the group consisting of a deoxynucleotide triphosphate
3 (dNTP), and a nucleotide triphosphate (NTP).

1 35. The intact charge-switch NP probe according to claim 34, wherein said
2 NTP probe is a deoxynucleotide triphosphate (dNTP).

~~copy~~

John G.K. Williams
Application No.: 09/876,375
Page 7

PATENT

~~COPY~~

1 36. The intact charge-switch NP probe according to claim 35, wherein said
2 deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of
3 deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate
4 deoxythymidine triphosphate and deoxyuridine triphosphate.

1 37. The intact charge-switch NP probe according to claim 34, wherein said
2 nucleotide triphosphate (NTP) is a member selected from the group consisting of adenosine
3 triphosphate, cytosine triphosphate, guanosine triphosphate and uridine triphosphate.

1 38. The intact charge-switch NP probe according to claim 31, wherein said
2 fluorophore moiety is attached to said terminal phosphate via a linker.

1 39. The intact charge-switch NP probe according to claim 38, wherein said
2 fluorophore linker is an alkylene group having between about 5 to about 12 carbons.

1 40. The intact charge-switch NP probe according to claim 38, wherein said
2 linker carries at least one positive charge.

1 41. The intact charge-switch NP probe according to claim 38 wherein said
2 linker carries at least two positive charges.

1 42. The intact charge-switch NP probe according to claim 29, wherein at least
2 one of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen atom
3 with a counter-cation associated therewith.

1 43. The intact charge-switch NP probe according to claim 29, wherein said
2 counter-cation is a metal ion.

1 44. The intact charge-switch NP probe according to claim 43, wherein said
2 metal ion is selected from the group consisting of Mg^{++} , Mn^{++} , K^{+} and Na^{+} .

1 45. A method for sequencing a nucleic acid, said method comprising:
2 providing a target nucleic acid, a primer strand, a polymerase, and a plurality of
3 NP probes;

~~COPY~~

John G.K. Williams
Application No.: 09/876,375
Page 8

~~COPY~~

PATENT

4 mixing said target nucleic acid, said sequencing primer, said polymerase, said
5 plurality of NP probes in a flowcell under conditions permitting target dependent polymerization
6 of said plurality of NP probes, thereby providing a polymerization product; and
7 separating the polymerization products by an energy field in said flowcell to
8 provide a sequence of said target nucleic acid.

1 46. The method according to claim 45, wherein the polymerization of said
2 plurality NP probes produces a plurality of phosphate detectable moieties.

1 47. The method according to claim 45, wherein said plurality of NP probes are
2 incorporate'd on said primer strand hybridized to said target nucleic acid using said polymerase,
3 thereby releasing a γ -phosphate with a detectable moiety attached thereto.

1 48. The method according to claim 45, wherein said energy field is an electric
2 field.

1 49. The method according to claim 48, wherein said electric field is a first
2 electric field applied in the transverse direction and a second electric field applied in the axial
3 direction.

1 50. A method for sequencing a nucleic acid, said method comprising:
2 providing a target nucleic acid, a polymerase priming moiety, a polymerase, and a
3 plurality of intact NP probes;
4 mixing said target nucleic acid, said polymerase priming moiety, said polymerase
5 and said plurality of NP probes under conditions permitting target dependent polymerization of
6 said plurality of NP probes, such conditions which are capable of providing a time sequence of a
7 plurality of phosphate detectable moieties;
8 separating by charge said plurality of phosphate detectable moieties from said
9 plurality of intact NP probes; and
10 detecting over time said plurality of phosphate detectable moieties to provide a
11 sequence of said target nucleic acid.

~~COPY~~

John G.K. Williams
Application No.: 09/876,375
Page 9

PATENT

~~COPY~~

1 51. The method according to claim 50, wherein said primer moiety is a hairpin
2 loop.

1 52. The method according to claim 50, wherein said plurality of phosphate
2 detectable moieties independently selected from the group consisting of PPI-Dye, a terminal
3 phosphate fluorophore moiety, a detectable moiety, charged groups, electrically active groups,
4 reporter groups, and combinations thereof.

1 53. The method according to claim 52, wherein said phosphate fluorophore
2 moiety is a used for a member selected from the group consisting of one-color sequencing, two-
3 color sequencing, three-color sequencing, four-color sequencing and combinations thereof.

1 54. The method according to claim 50, wherein said polymerase is
2 immobilized in single molecule configuration.

~~COPY~~